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Whole-mount Xenopus Immunostaining Protocol

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An abbreviated version of this protocol was published in eLIFE in Apr 2020

Chromatin accessibility dynamics and single cell RNA-Seq reveal new regulators of regeneration in neural progenitors

DOI: 10.7554/eLife.52648

Detailed protocol

Whole Mount *Xenopus* Immunostaining Protocol

Adapted from Helen Willsey, modified after Peter Walentek and Susanne Bogusch

Reagents:
-1x MEM with 3.7% formaldehyde
-CAS-Block (Invitrogen #00-8120)
-1X PBS
-1X PBT (PBS + 0.1% Triton X-100)
-1X bleach: per ml= 50uL Formamide, 40uL Peroxide, 910uL PBS

Protocol:

1. Fix tadpoles in 1x MEM with 3.7% formaldehyde 30min-1hr RT. (NO LONGER THAN 1hr)
2. Optional: bleach tadpoles for 2hr RT under light box.
3. Permeabilize by washing 3x20' RT PBT.
4. Block in 10% CAS-Block in PBT 1hr RT.
5. Incubate in 1^{o} antibody diluted in 100% CAS-Block O/N 4^{o}C.
6. Wash 3x10' PBT RT.
7. Block again in 10% CAS-Block in PBT 30'.
8. Incubate in 2^{o} antibody diluted in 100% CAS-Block 2hr RT.
9. Wash 3x10' PBT RT.
10. Optional: Incubate in Phalloidin-647 diluted 1:40 in PBT 30' RT.
11. Wash 3x20' PBS.
12. Mount with Vectashield + DAPI.

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How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Wills, A. (2020). Whole-mount Xenopus Immunostaining Protocol. Bio-protocol Preprint. bio-protocol.org/prep620.
2. Kakebeen, A. D., Chitsazan, A. D., Williams, M. C., Saunders, L. M. and Wills, A. E.(2020). Chromatin accessibility dynamics and single cell RNA-Seq reveal new regulators of regeneration in neural progenitors. eLIFE. DOI: 10.7554/eLife.52648

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